(B) — Evaluation of Diagnostic Microbiologic Studies

Susan J. Hadley*

Fifteen years ago, many persons thought that the use of broad-spectrum antibiotics would greatly diminish the need for microbiologic procedures. Figure 1 shows what actually has occurred. The chart indicates the number of procedures performed by a diagnostic bacteriology laboratory in a large general hospital in New York. This hospital has had less than a 10 per cent increase in beds during the period shown. The great increase in number of procedures reflects a variety of influences, among them a change in attitude toward the need for bacteriological studies. Today, it is apparent that the specific bacteria, virus or parasite causing disease must be definitely identified whenever possible. With the great increase in the use of laboratories and the growing dependence on them, it has become more important for the doctor to be able to analyze results wisely. The sessions that follow this one will include discussions of bacteriological, viral and parasitic disease. Tonight, therefore, we shall consider some problems in the evaluation of bacteriologic studies.

Evaluation must begin by considering the adequacy of the specimen submitted to the laboratory. Often, diagnoses are ruled out on the basis of studies performed on totally inadequate specimens. The prime responsibility for the specimen is yours. It should not be delegated to an aide. Listed in Table I are considerations important in the selection of material for microbiologic examination.

1. Before one can decide on the specimen to be cultured, the diagnostic possibilities for the patient must be outlined. This is required in order to determine the type of material best for culture. For example, in the patient with aseptic meningitis it would seem logical to send spinal fluid to the laboratory but actually, a stool specimen is preferable in polio, Coxsackie and other enterovirus infections and should, indeed, be accompanied by an acute phase serum. When one is not certain what body fluid is best to send for study, it is wise to consult a textbook or call the laboratory for advice.

* Clinical Assistant Professor of Medicine, Cornell University Medical College.

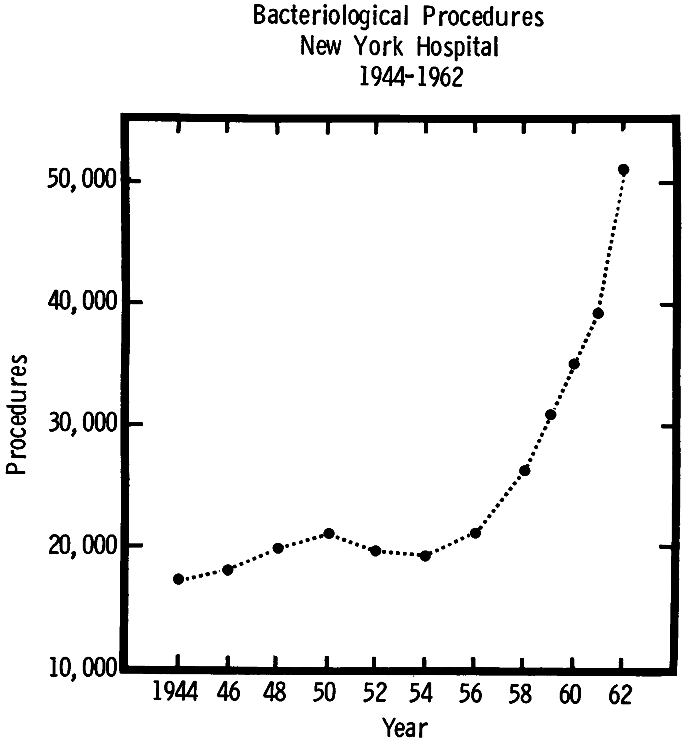

Fig. 1

2. The choice of the specimen will also depend on the stage of the disease. This is demonstrated in typhoid fever, for example, where the bacillus is most often found in the blood during the first week of illness, while during the second and third weeks it is most often found in the stool. Similarly, in leptospirosis after the first week of the illness, the organism is practically never in the blood but may be found in the urine.

3. Another consideration in ascertaining the adequacy of the submitted specimen is its preservation. In general, material sent to the laboratory for culture should be freshly obtained and delivered during laboratory hours. It has been shown repeatedly that pathogenic enteric organisms disappear rapidly from stools. Specimens after two to three hours without preservation may no longer yield organisms easily isolated on the same specimen when fresh. This is true of the Salmonella group,

TABLE I.—CONSIDERATIONS IN THE SELECTION OF SPECIMENS FOR MICROBIOLOGIC STUDY

Diagnostic possibilities
Choice of specimen
Stage of disease
Preservation of specimen
Absence of antibiotics
Consultation with the laboratory

and even more true of Shigella which are cultured best when plated at the bedside.

If the location or type of medical practice make it difficult or impossible to get specimens to the laboratory promptly, one must investigate the kind of preservatives available and determine those preferred by the laboratory of your choice. It will also be necessary to become familiar with the temperatures required for preservation. Most specimens for virus isolation must be kept at -70°C. until ready for study. Neisseria on the other hand, in contrast to other bacteria, die off rapidly at refrigerator temperatures and must be kept at body temperature.

4. More often than many physicians realize, a golden opportunity is lost by the one- or two-dose administration of antibacterial agents used before specimens are obtained. Even a single small dose of penicillin given less than an hour before obtaining a specimen of sputum from the pneumonic patient, may make isolation of the pneumococcus impossible. Organisms may be seen and even typed, but growth may be very slow, or not occur at all. In the bacteremic or septicemic patient, this is equally true. The tragedy is that the drug being used is often completely ineffective in treating the patient although completely successful in inhibiting growth on bacteriologic media. Regretfully, the situation is seen all too often in the child with bacterial meningitis and the patient with subacute bacterial endocarditis. It may take one to two weeks off antibiotic before a positive blood culture can be obtained.

5. In no other area of laboratory medicine is it so important for the doctor to communicate with the diagnostic facility. In the ordinary course of events, there are a limited number of bacteriological ap-

TABLE II.—DISTRIBUTION OF MICROORGANISMS ON BODY SURFACES†

Organism	*Skin*	*Pharynx*	*Genit.*	*Intest.*
Staph.	+	+	*	*
Alpha Strep.	*	++	+	+
Actinomyces		+		*
Escherichia	*		+	++
Clostridium				+
Bacteroides		*	*	++
Mycobacterium			+	

† Modified from a chart by T. Rosebury in *Bacterial and Mycotic Infections of Man,* edited by R. J. Dubos, 2nd ed., Philadelphia, J. B. Lippincott Co., 1952, p. 693.
* Irregular. + common or constant. ++ most numerous or characteristic

proaches possible on an average specimen. These are selected on the basis of likely possibilities. Thus, in the urine where Gram-negative rods are the most commonly isolated microbes, one does not prepare the specimen to look for Leptospira which require a media rich with serum incubated at temperatures lower than 37°C. Neither can the laboratory be expected to use routinely media suitable for the growth and isolation of the Brucella, *B. tularense* or Eaton Agent. All require special media. Eaton Agent, which has been shown to be the cause of 90 per cent of cold agglutinin-positive atypical pneumonia, was thought to be a virus. This year it has been shown that it can be cultured on artificial media. None of these organisms would have a chance of being isolated unless the clinician alerted the laboratory. Having been alerted, the laboratory can choose media suitable for isolation of these less usual organisms. If it cannot do so, it will refer the specimen to a laboratory which can.

The first step, then, in evaluating the laboratory report is consideration of whether the specimen studied was adequate.

After results of cultures are reported, an analysis of organisms isolated is required. It is necessary to bear in mind the normal flora of the area from which the specimen was taken. Table II, modified from a chart of Rosebury, illustrates a few examples which emphasize this point. Actinomyces in sputum may only reflect the presence of the organism in the normal pharynx which contaminated the sputum as it passed the area. One must remember this in considering the organism as the cause of lung disease. When acid-fast smears of urines are positive, one must always consider the possibility of these representing non-pathogenic mycobacteria from the external genitalia. Clostridia are present in large numbers in feces and are often only contaminants when isolated from wounds that might be in contact with fecal material. More than once, I have seen a clinician greatly concerned that his patient had a gas gangrene infection when actually the organism isolated was only a contaminant.

Increased interest has arisen regarding quantitation of the number of bacteria present in specimens. Urine cultures have received special attention recently because of the need to evaluate the clean-catch specimen. Beeson and others have demonstrated that even a single insertion of a urethral catheter may establish a chronic urinary tract infection. This unfortunate circumstance results from introducing infected material into the bladder by insertion of the catheter through the bacteria-containing external urethra. The clean-catch specimen was devised to avoid catheterization whenever possible. It is simply a mid-stream specimen obtained after cleansing the external genitalia in either the male or female subject. This type of collection will not introduce organisms into the bladder, but the urine may contain some bacteria from the external urethra and genitalia. The old method of culturing urine in liquid media will not differentiate these contaminants from those causing disease. It is easy, however, to quantitate the number present by using pour plates or by streaking urine on plates with calibrated bacteriologic loops. The criterion of 10^5 (100,000) or more bacteria/ml. of urine has been generally accepted as an indication of urinary tract infection. An interesting fact that has come from the regular use of quantitative studies of urine has been the number of cases of significant bacteriuria that are asymptomatic. In some series it has been as high as 10 to 20 per cent.

Some laboratories are using quantitative studies of sputum and wound exudates to follow the course of disease and to study the effectiveness of therapy. This, however, is generally limited to the research laboratory.

In conclusion, a few words about the interpretation and use of antibiotic sensitivity tests are appropriate. Having isolated an organism considered to be the cause of illness, the next step is usually to use the so-called disc sensitivity tests in order to select the antibiotic for treatment.

TABLE III.—EVALUATING DISC SENSITIVITY TESTS

Consider:

1. Concentration of disc
2. Diffusibility of antibiotic
3. Characteristics of antibiotic in body
   Absorption, Excretion
   Distribution, and Toxicity
4. Action of drug—bacteriocidal or bacteriostatic
5. Need for precise quantitation
6. Experience with drug in the patient

These tests are in common use and for the most part serve as a suitable qualitative guide to treatment. There are a few considerations in their use to which I would like to draw your attention.

Antibiotic discs are provided to laboratories in a variety of concentrations. In some cases the disc may be impregnated with lower concentrations of drug than can be obtained in the patient. A good example of this is *Proteus mirabilis* infections where, in contrast to all other Proteus species, high concentration of penicillin (in the range of 50 units/ml. of serum) will effectively treat the infection. Such levels can be obtained by high-dose intravenous therapy. The discs used ordinarily are in the range of 2 units so that the test may indicate that the organism is resistant, while in fact it is not. Some antibiotics diffuse into the media very poorly so that even small halos about the discs represent effectiveness of the agent. These may be misinterpreted, so that the organisms are reported to be resistant. This situation pertains to bacitracin, polymyxin, colistin and neomycin. Final selection of the drug often depends, however, on factors not directly related to the laboratory tests. The disc, for example, will not reflect the degree of absorption or destruction of a drug nor its distribution in the body or toxicity. Nor does the disc method indicate whether the drug has the potential to kill or merely to inhibit growth. For the most part, whenever a serious infection is present, a bacteriocidal drug is the one of choice. Tube dilution tests, although not available for wide use, can give more precise sensitivity studies and determine at what level the drug is bacteriocidal. In the treatment of bacteremia this type of data is often essential. Lastly, it is wise to point out that, in the selection of the best drug for the patient, previous experience is invaluable. In the treatment of Salmonella infections, although sensitivity tests would indicate a variety of drugs to be useful, experience has shown that chloramphenicol is the drug of choice. In enterococcal subacute bacterial endocarditis, the combination of penicillin and streptomycin is the one of choice, although studies may show the organism to be resistant to both. Bearing all this in mind, final success depends on the response of the patient at hand.

At best, specific diagnoses are slower than optimal. One wishes that it were possible to grow and identify bacteria in a few hours. Many microbiologists are interested in this problem but to date research has been without startling success. The use of fluorescent-labelled antibody may be helpful in this area. The technique of staining smears of throat exudate with flourescent antibody has been shown to compare well with the results of cultures when looking for Group Aβ hemolytic streptococci. In general, however, to date immunofluorescence has had limited usefulness in the general diagnostic laboratory and other techniques will need to be sought to speed diagnostic studies.

Conclusion

In order to gain the greatest value from bacteriological studies, one should select the proper specimen, deliver it promptly to the laboratory, analyze the report considering the normal flora, and use an antibiotic sensitivity test, realizing that, in addition to disc sensitivity tests, the experience of others will be valuable in selecting the best drug for treatment.

COLLATERAL READING LIST

1. *Bacterial and Mycotic Infections of Man,* R. J. Dubos, ed. Philadelphia, J. B. Lippincott Co., 2nd edition, 1952; 3rd edition, 1958.
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4. *Viral and Rickettsial Infections of Man.* Th. M. Rivers and F. L. Horsfall, eds. Philadelphia, J. B. Lippincott Co., 1959.